RESEARCH ARTICLE

The effect of acidification process on camel milk total casein coagulation and fractionation

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Caseins are the most important proteins in camel milk in terms of quantity and quality. However, the process of their sedimentation has always been a major obstacle to converting camel milk into derivative products such as cheese and yoghurt due to its physicochemical properties that make the acidification a very complex process. Throughout this study, the effectiveness of using Acetic acid (HOAc) 10% with its conjugate base, sodium acetate (NaOAc) 10%, in separating total caseins from whey, compared to hydrochloric acid (HCl), is demonstrated. The influence of the acidification process on the curd texture and on the urea fractionation method was also investigated by separating different casein fractions on Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE). The precipitated curd texture is firm, manipulable with a high consistency, and the peaks of each fraction on electrophorese diagrams seem to be clear and evident without any contaminant peptides from whey when using HOAc 10% with its conjugate base. This acidification process allows obtaining a significant number of total caseins of strong structure and firmness required to produce dairy products of good quality and desired appearance in order to be transferred to industry level and to be accepted by consumers.On the other hand, if it is approved, this process could be interesting in term of costs and time compared to traditional camel milk coagulation processes.

Keywords: acidification; camel milk; casein coagulation; SDS PAGE; urea fractionation.

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Introduction

Milk is the main and only source of nutrition for newborns [1]. It is also considered a complete food for children and adults. It provides the necessary nutrients such as proteins, carbohydrates, fatty acids, minerals, vitamins, growth factors and active immune molecules [2-4]. Currently, milk and dairy products represent an important social territorial and economic pillar in international trade [5]. The consumption of milk represents a heritage and a cultural inheritance in some regions. Moreover, for the desert inhabitants of arid and semi-arid regions, camels play a major role in supplying them with milk of high nutritional quality [6-8], under hostile environmental conditions of high temperatures, drought and lack of pastures [9, 10], a milk that meets their requirements and daily needs, due to its containment of key nutrients and natural protective molecules such as enzymes and antibodies, making it a unique compound [11]. Besides, it does not cause

allergic reactions in children and adults compared to cow's milk [12, 13].

Camel milk contains a high proportion of proteins that are divided into soluble proteins and caseins (CNs) [13, 14]. The proportion of CNs in camel milk may reach 87% of total proteins, this makes it the most important macromolecule quantitatively and qualitatively [15, 16], as it contains a large proportion of the essential amino acids especially Tryptophane, Cysteine, and Lysine residues [17]. Total casein (TCN) contains three fractions including α-, β-, and κ-CN [16, 18] with the molecular weights of 27.6, 23.8 and 22.4 KDa, respectively [19]. The process of acidifying camel milk in order to separate the TCN from the serum, affects the yield and purity of caseins [20], it has received wide attention whether for economic purposes or for an in-depth study of these molecules. Camel milk is usually consumed fresh and is rarely converted into derivative products, unlike cow milk [21]. What hinders the process of converting it, is the difficulties that accompany the coagulation process, which is essential for the production of dairy products [22]. CNs are the main component of the dairy product manufacturing process, where their physical and chemical properties control the quality and cost-effectiveness of the production of dairy products [23, 24]. Digestive enzymes and antibodies negatively affect the biological acidification process [25]. Whereas the physicochemical properties of camel milk such as the high ratio of whey proteins (WP) to CNs, the large casein micelle size in the camel milk [22] and the lack of k-CN and β-lactoglobulin interactions hinder the classic chemical acidification process [22, 26, 27].

Since the acidification process of camel milk is considered a key stage that determines the rheological and physicochemical properties of the resulting curd, which in turn determines the quality and the appearance of dairy products, and because camel milk shows extreme resistance to precipitation by acidification, this comparative study aims to choose the optimal acidification method that leads to obtain firm curd with a stable structure and high consistency, which facilitates the process of converting it into high quality dairy derivatives. Throughout this study, a comparison of the effects of camel milk acidification between dilute HCl method and HOAc with its conjugate base, NaOAc, method was investigated. The influence of acidification process on the curd texture and on urea fractionation method were also confirmed by separation of different casein fractions on Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE).

Materials and Methods

During the process of caseins sedimentation and their fractionation process, appropriate chemical solutions were used and undistorted sedimentation and separation techniques were relied upon, in order to preserve the initial structure of the proteins.

Sample preparation

The biological sample is fresh camel milk. The camel ''*Camelus dromedarius L*'' belongs to the Sahrawi or "Mihiri" breed, a hybrid of the two strains of Al-Chaambi and wled Sidi Al-Sheikh. 15 camels of ten years old from commercial farm (Aissa's farm, Bir-Naam, Biskra, Algeria) were milked. Camel milk was obtained by manual milking in the afternoon at 4 pm. Obtained samples were mixed immediately and the temperature was reduced to 4°C. The sample was transferred to the laboratory where it was kept for one night at an estimated temperature of 4°C.

Preparation of skimmed camel milk

Prior to the total defatting process, the temperature of the sample was raised in a water bath to 36°C. The sample was defatted by centrifugation at 6,000 rpm for 15 minutes at 36°C. The samples were cooled down. The supernatant was separated, and the skimmed milk was obtained.

Acid casein preparation "the acidification process"

In the first method, 1 M HCl was used to reduce the pH to 4.6 according to the modified method of Shammet [28]. The precipitated total caseins (TCN) were then separated from the serum by centrifugation at 6,000 rpm for 15 minutes at 15°C. As for the second method, according to Mohamed's approach [29], in the first stage, HOAc was added to the completely skimmed milk by 10% relative to the initial volume at 37°C, and then in a second stage, NaOAc was added to the previous solution by 10%. TCN were precipitated and obtained by centrifugation at 5,000 rpm for 15 minutes at 15°C. In both cases, the obtained TCN are washed several times with distilled water.

Total Caseins urea fractionation

Fractionation of TCN was carried out according to the modified urea fractionation method [30]. TCN were dissolved in a double volume of 10 M urea solution. After mixing, the solution was diluted by adding double volume of distilled water. The pH was raised to 7.5 by the addition of 1 M NaOH, and then distilled water was added until the solution became clear. The pH was reduced again to 5.0 by the addition of 1 M HCl. The mixture was centrifuged at 5,000 rpm for 15 minutes at 15°C. The precipitate containing the α-CN and κ-CN fractions was obtained, while the β-CN fraction remained in the supernatant. After salt saturation of the supernatant by the addition of sodium chloride (NaCl) gradually while stirring, β-CN fraction was precipitated and separated by centrifugation at 5,000 rpm for 15 minutes at 15°C. All isolated fractions were dialyzed against distilled water using dialyze membranes to remove different reagents before recovering in appropriate tubes tightly closed and reserved at 4°C.

Casein fractions characterization by SDS–PAGE

The operation was carried out according to the method described by Laemmli [31]. The homogenized samples were incubated at 98°C for 5 mins. After centrifugation, 100 µL of the supernatant of each sample (0.1 mg) was mixed with 4 mL of freshly made protein solubilization buffer (4.25 mL of a stock solution (7.5 mL of tris-HCl (pH 4.6), 12.5 mL of distilled water, 2 g of SDS, 20 mg of methylene blue, 10 mL of glycerol), 0.75 mL of 2-β-Mercaptoethanol, 10 mL of distilled water) and loaded on the gel consisting of a 12% separation gel and 2.7% concentration gel with the dimensions of 100 mm × 100 mm × 1.5 mm. The electrophoresis was performed under a constant current of 200 V and 25 mA intensity for approximately 4 hours by using Mini-PROTEAN® Vertical Electrophoresis Cell (Bio-Rad, Hercules, California, USA) along with a low range molecular weight (6 – 45 KDa) standard protein marker (Sigma-Aldrich, Saint-Louis, Missouri, USA). After electrophoresis, the gel was removed and placed in the staining solution composed of 31.5 mL of Coomassie blue R250, 125 mL of 50% EtOH, and 50 mL of 10% HOAc adjusted to 500 mL with distilled water. After 24 hours staining, the gel was replaced in discoloration solution consisting of 125 mL 50% EtOH and 50 mL of 10% HOAc adjusted to 250 mL with distilled water. The gel was then visualized, and the image was captured by using Sony DSC-HX90 camera.

Results and discussion

SDS-PAGE was carried out under undistorted conditions where the peptides kept their initial structure intact. The different fractions were recognized after comparing their molecular weights with standard protein marker molecular weights. The results showed that, in the first method, the TCN was coagulated using dilute HCl. There was no casein fractionations of α-, β- and κ-CN bands appeared in lanes B1 and B2, instead there were many bands of contaminant peptides from whey precipitated during the acidification process (Figure 1). As for the method which the TCN was coagulated by using HOAc and its conjugated base, α-, β- and κ-CN bands were obviously clear with no visible contaminant peptide bands, which indicated that urea fractionation method was efficient and there was no other precipitated peptides during acidification process. Therefore, acidification process directly influenced TCN coagulation and its fractionation.

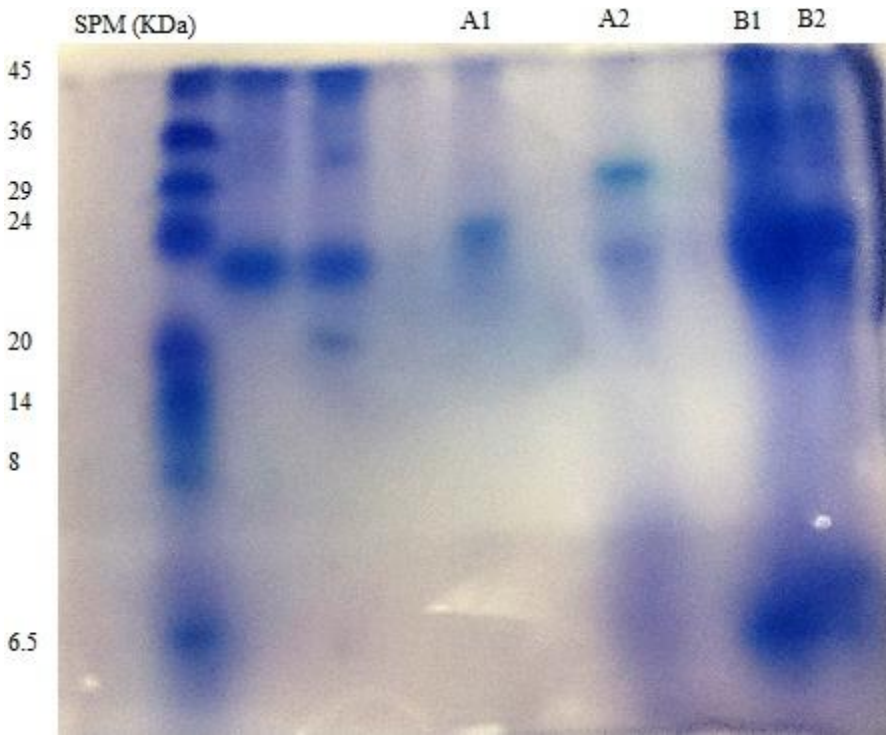

Figure 1. SDS-PAGE of the two curds obtained from casein urea fractionation method. Lane SPM: standard protein marker (KDa). Lane A1: β-CN fraction. Lane A2: α- and κ-CN fractions. Lane B1: β-CN fraction. Lane B2: α- and κ-CN fractions.

In the first method when using dilute HCl, it decreased the size of casein micelles during acidification process [32-33]. The size diminution was due to the demineralization of micelles. The total net charge decreased, and the micelles started to become closer to each other [34], small number of amino groups were then available on the surface leading to lower interactions with HCl ions [35], which explained the resistance to coagulation, the weakness of texture, and the thinness of consistency. Even diluted, HCl reduced the pH of fresh camel milk quickly and modified irreversibly the surface composition of camel milk casein micelles [36], which resulted in the precipitation of whey proteins and the denaturation of casein micelles. In this case, urea concentrated solutions did not react with casein micelles the same way as in the differentiated solubility using concentrated urea solutions, which explained the inefficiency of urea fractionation method on camel milk TCN, confirmed by SDS-PAGE and the appearance of contaminant peptides. While in the case of using a buffer solution composed of HOAc with NaOAc as a conjugate base during acidification process, the pH of fresh camel milk reduced gradually, which affected the stability of camel milk TCN micelles by neutralizing its negative charges without affecting the composition [37, 38]. The conjugate base enhanced the electrostatic repulsions between micelles, leading to the loose of some fractions and the increasing of casein micelles size [33], which made the coagulation possible and rapid at the desired pH. The curd was then firm and consistent.

Conclusion

The process of camel milk acidification to separate the total casein from the serum is a key stage that affects the quantity and quality of the coagulated caseins. Acidification process using organic acid with its conjugate base to coagulate camel milk total caseins has confirmed its efficiency in the obtaining of the desired curd

texture and composition, ready to be used by camel milk industry to produce dairy products of good appearance and high quality. It is Important to test the effect of other organic acids such as citric acid and lactic acid on camel milk acidification process to consolidate the results of this study to improve the cost-effectiveness and to study the feasibility at an industry level.

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